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Protective Effect of Vitamin E on Endosulfan Induced Testicular Toxicity in Swiss Mice (*Mus musculus*)

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Abstract

The present study was aimed to evaluate the role of vitamin E against the testicular toxicity induced by endosulfan. 8-10 weeks old, 24 healthy male animals were randomly selected and equally divided into 4 groups. Group I served as control group (C); group II endosulfan group (ES); group III vitamin E group (VE) and group IV vitamin E plus endosulfan group (VE+ES). Group C animals were given only vehicle the olive oil; in groups ES and VE+ES endosulfan was administered orally at a dose of 2.45 mg/kg b.w.; in groups VE and VE+ES, vitamin E was administered orally at a dose of 50 mg/kg b.w. In group VE+ES, vitamin E was administered 1 hour prior to endosulfan administration. All treatments were given continuously for 15 days. Endosulfan intoxication resulted in decreased testis weight tend severe histopathological changes which included shrunken and distorted seminiferous tubules and atrophy in the tissue. It was observed that administration of vitamin E minimized the endosulfan induced damage. Thus it can be concluded that pretreatment with vitamin E can alleviate the damage caused to testis by endosulfan.

Key Words: Vitamin E; Endosulfan toxicity; Testis; Mice

Introduction

Endosulfan is a potent organochlorine pesticide, under the cyclodiene subgroup that has emerged as a leading chemical used against a broad spectrum of insects and mites in agriculture and allied sectors. India is the world's largest producer and user of endosulfan (1) and it is one of the most commonly used pesticides in India (2). Studies indicate that brain, liver, kidney, immune system and testes are the target organs bearing major impact of endosulfan toxicity (3,4). Endosulfan is known to damage the endocrine system, nervous system, circulatory, reproductive, respiratory and excretory systems and developing foetus (5-8).

Traces of endosulfan have been detected in a great number of supermarket goods including vegetables, seafood and even spices (9). Endosulfan is readily absorbed by humans via the stomach, lungs and through the skin. It also causes endocrine disruption (10).

Endosulfan is known to cause possible damage to reproductive system of male rats (10).In recent years, there has been growing concern about toxicity of pesticides, on the male reproductive system (11,12). It has been reported that endosulfan exposure resulted in delayed sexual maturity in boys in Kerela, India where endosulfan was used on cashew plantations (13).

Vitamin E is one of the most potent lipid soluble free radical scavenger that limits lipid peroxidation initiated by free radicals in the testicular microsomes and mitochondria (14).Vitamin E is a powerful lipophilic antioxidant vital for the maintenance of mammalian spermatogenesis (15). It also reverses the detrimental effects of oxidative stress on testicular function mediated by different agents (16-19)

Histopathological analysis is regarded as a realistic biomarker in toxicological studies (20). Histopathological studies are useful to evaluate the pollution potential of pesticides since trace levels of pesticides, which do not cause animal mortality over a given period, are capable of producing considerable damage in the tissues (21). Any toxicant either acts directly on the cell or most frequently causes cytotoxicity by modulating the cellular microenvironment. The cells in turn respond histopathologically by degeneration, proliferation, inflammation and repair (22).

In vivo histopathological alterations in the testis due to endosulfan intoxication and the protective role of vitamin E seem to be lacking in the literature. Keeping in mind the above facts, the present study was designed to have an insight into histological analysis regarding the extent of damage of the testicular tissue due to endosulfan toxicity and its subsequent mitigation by Vitamin E.

Materials and Methods

Chemicals

Endosulfan (CAS No. 115-29-7 and purity 99.9%) was obtained from Shree Pesticides India Ltd. Vitamin E was obtained from Himedia, India. All other chemicals and solvents used were of analytical grade.

Animals and Treatment

Male Swiss mice numbering 24, weighing 30 ± 5 gms and 8- 10 weeks old were used in the present study. The animals were maintained on sterilized rice husk bedding in

polypropylene cages and kept at a temperature of about 23±3 ºC with 12±1 hour light/dark cycles. The animals were fed on standard pellet diet (Pranav Agro, Baroda). Food and water were given *ad libitum*.

This experimental study was approved by the Institutional Animal Ethics Committee. The handling of the animals was according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Govt. of India.

Dose Selection

The dose for endosulfan was selected after conducting pilot experiments in our laboratory. The LD50 for endosulfan was found to be 7.35 mg/kg b.w. (23). The dose selected for endosulfan was one third of the LD50 i.e., 2.45 mg/kg b.w. and the duration of treatment was 15 days. The dose selected was lower than which was used in earlier studies (24)

The doses for antioxidants were calculated keeping in mind the doses prescribed for humans. The dose of vitamin E selected was 50 mg/kg b.w.

The doses were prepared by dissolving in olive oil.

Experimental Protocol

A sub chronic study was done for 15 days and oral route of dose administration was chosen for all the treatments. The mice were divided into four groups with minimum of 8-10 animals in each group.

Group I	Control (C) group (given olive oil)
Group II	Endosulfan (ES) group (given 2.45 mg/kg b.w. dose of endosulfan dissolved in olive oil)
Group III	Vitamin E (VE) group (given a vitamin E @ 50 mg/kg b.w.)
Group IV	Vitamin E and endosulfan (VE+ES) group (given vitamin E @ 50 mg/kg b.w plus endosulfan @ 2.45 mg/kg b.w.)

In group IV, the vitamin E was administered 1 hr prior to endosulfan administration.

Histological Preparation

The mice were sacrificed by cervical dislocation at the end of the scheduled period of 60 days and 24 hrs after the last dose treatment. After dissection both the testis were weighed and the left testes was fixed in 10% formalin solution. After 18 to 24 hrs of fixation the testes was further processed for paraffin embedding. Serial sections of 5-7 µm thick were cut using a rotary microtome. The deparaffinized sections were routinely stained with haematoxylin and eosin, examined and photomicrographed.

Result

Effect on testis weight

A significant reduction in the testis weight was observed in the endosulfan intoxicated animals as compared to the control (C) group. Endosulfan intoxication resulted in a significant decrease ($P< 0.01$) in the testis weight as compared to control (C) group. Pretreatment with vitamin E in group VE+ES significantly alleviated ($P< 0.01$) the testis weight as compared to group ES (Table 1).

Table 1—Changes in the average weight (g) of body weight in control (C), endosulfan treated (ES), Vitamin E (VE) and vitamin E plus endosulfan treated (VE+ES) groups [Values are Mean ± SD].

Group	Average body weight (mg)
C	119.17 ± 5.84
ES	97.50 ±5.24**[a]
VE	125.00 ± 4.47
VE + ES	115.83 ±3.76**[b]

** $P< 0.01$ * $P < 0.05$; a: group ES compared with group C; b: group VE+ES compared with group ES

C: Control group; ES: endosulfan treated group; VE: Vitamin E group; VE+ES: Vitamin E plus Endosulfan treated group

Histopathological findings

Normal architecture was seen in the histological sections of testis of control group (C) (Plate 1, Fig.1). Healthy and normal seminiferous tubules were observed. The seminiferous tubules manifested all the cells of the spermatogenetic series such as spermatogonia (SG), spermatocyte, spermatids, spermatozoa (S), and sertoli cells (SC). The lumen of the tubules was occupied by mature spermatozoa. Healthy blood vessels (BV) and Leydig cells (LC) were present in the interstitium.

Endosulfan intoxication resulted in the adverse changes in the testicular tissue. Sections of testis from endosulfan group (ES) showed degenerative changes. In few tubules the lumen was completely filled with edematous fluid (Plate 1, Fig 2). Sloughing of the germinal cells lining the seminiferous tubules was a common feature among a number of tubules (Plate 1, fig.3). Majority of the seminiferous tubules were shrunken and had a distorted appearance (Plate 1, Fig. 4).

There was depletion and hypertrophy of germ cells. Lumen was enlarged and binucleated (BN) and trinucleated (TN) cells could be seen in the lumen alongwith dead cells (DC) and spermatogonia.(Plate 1 , Fig, 5; Plate 2, Fig, 6&7)) Oligospermia and complete absence of spermatozoa from the lumen was notable. Plate 2, Fig. 8 shows a giant cell (GC) with fusiform nucleus. Leydig cells and blood vessels were sparse in interstitium.

In group VE (Plate 2, Fig.9) the testis revealed normal structure as observed in group C.

In group VE+ES (Plate 2, Fig. 10) sections were quite similar to those of group C and the pathological changes like giant cells, multinucleated cells, enlarged lumen, distorted seminiferous tubules as observed in group ES were not seen. The structure was more or less restored.

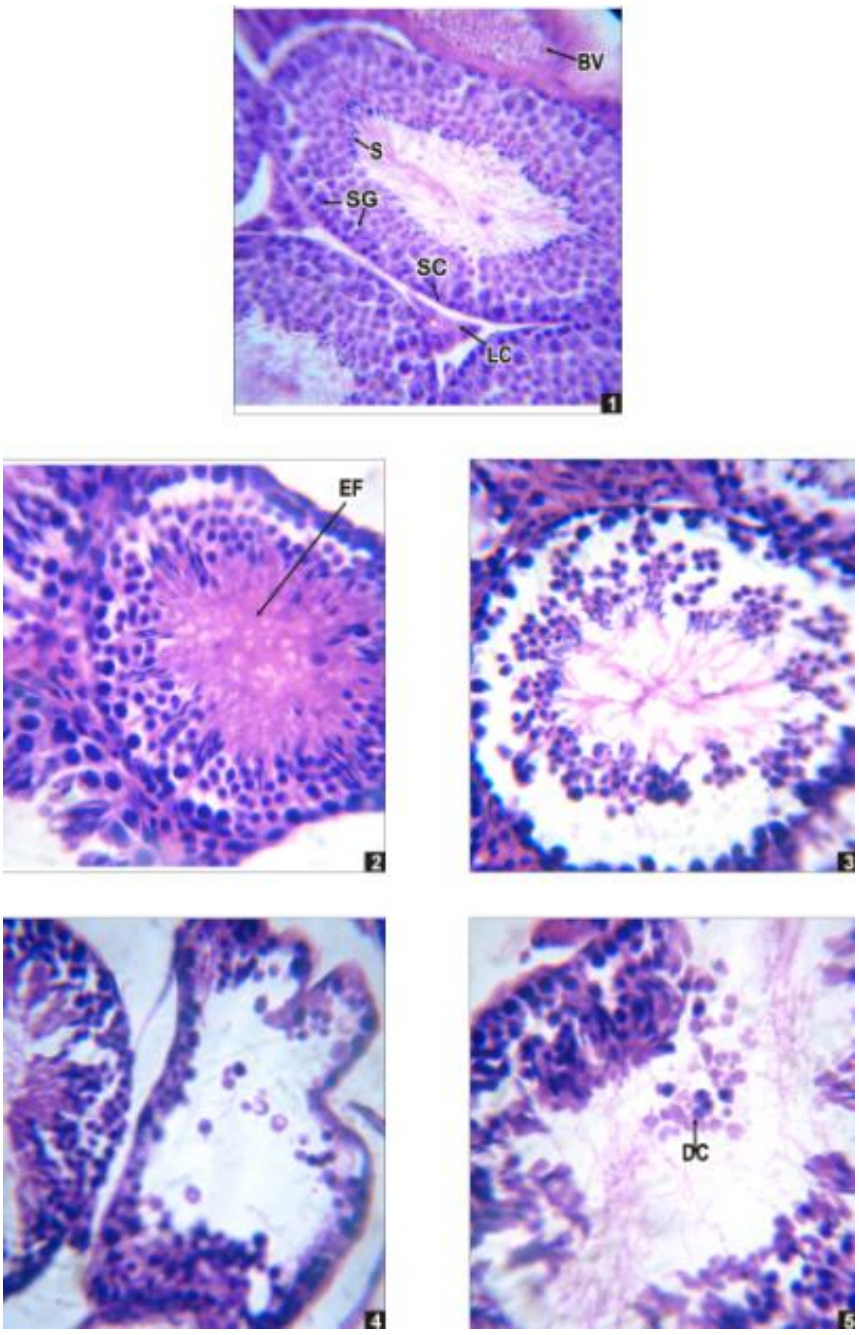

Plate 1: — Histological sections of testis stained by H & E at 400 ×. (Fig. 1)-Section of testis of control group showing normal seminiferous tubules with all the cells of spermatogenetic series. Spermatogonia (SG), Sertoli Cells (SC), Spermatozoa (S), Leydug Cells (LC)and Blood Vessels (BV) can be seen.

Endosulfan treated group [Plate 1, Fig. 2 – 5 and Plate 2 , Fig. 6 – 8] (Fig. 2)- shows edematous fluid (EF) in the lumen (L); (Fig. 3)-Dislodging and sloughing of the germ cells ; (Fig. 4)- Distorted seminiferous tubules (ST) with loss of germ cells and complete loss of spermatogonia; (Fig. 5)- Dead cells (DC) and germ cells are seen in the lumen;

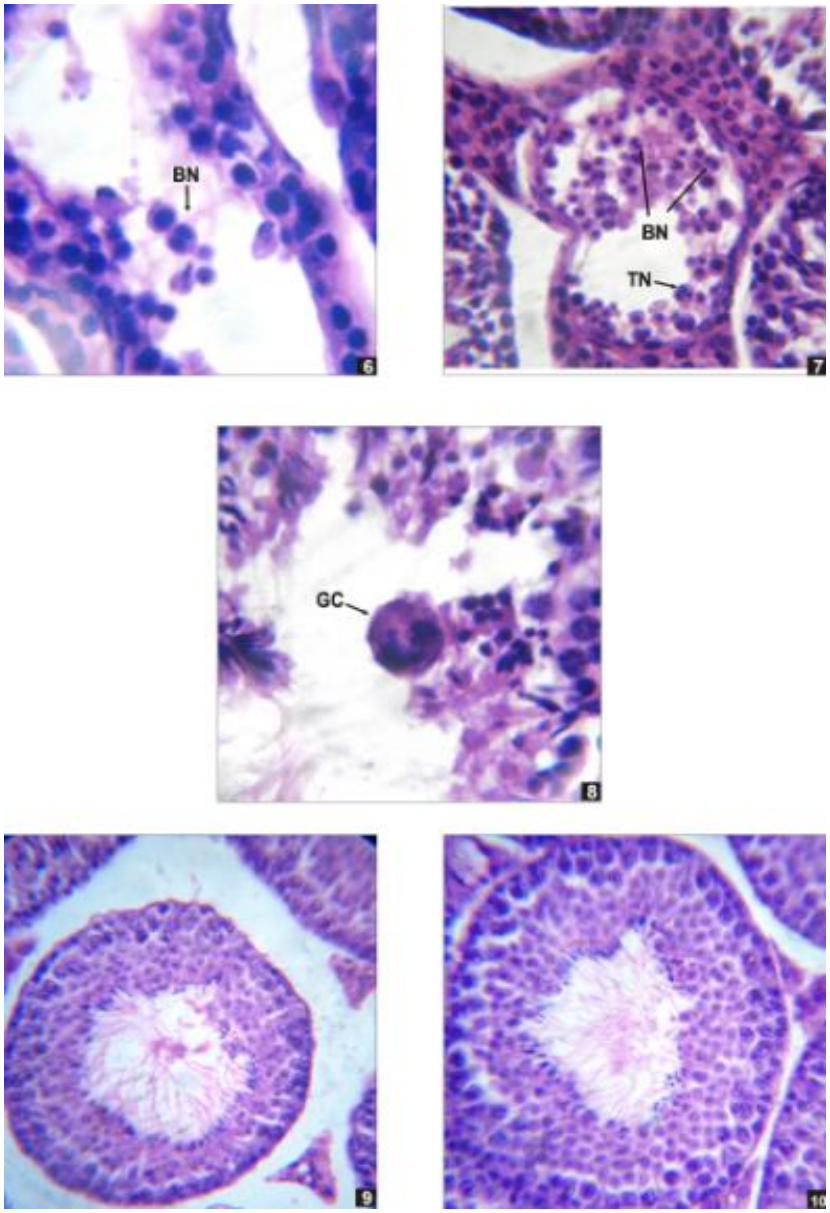

Plate 2 : (Fig. 6) – Binucleated cell (BN) is seen in the lumen; (Fig. 7)- Shrunken seminiferous tubules showing bi- and tri- nucleated (TN) germ cells; (Fig. 8)- A giant cell (GC) with a fusiform nucleus is seen; (Fig. 9) Section of vitamin E treated group showing normal seminiferous tubules; (Fig. 10)- Section of testis of vitamin E plus Endosulfan treated group showing features close to normal structure.

Discussion

Drastic changes were observed in the histology of testis after endosulfan treatment for 15 days. Occurrence of giant cells (25) and multinucleated giant cells (26-28) in the lumen as observed in the present study have been reported in a number of earlier studies. Such changes may be attributed to failure of cytokinesis and hypertrophy (29). Vacuolations observed are similar to earlier reports (30).

The decreased number of Leydig cells in interstitium in the exposed groups is in accordance with earlier reports on testicular toxicity due to other organochlorine pesticides (29). The inhibition of testicular androgen biosynthesis (31) could be attributed to the present observation.

Oligospermia as observed in the present study is a remarkable observation of testicular toxicity as also reported in earlier studies (29, 31-33)

Dead cells in lumen and the decreased number of germ cells may be due to cytotoxic (34) and apoptotic (24) effect of endosulfan. The reduction and degeneration of germ cells can be a possible reason for the endosulfan induced disruption of the spermatogenic (7) and steroidogenic cycle (31,33). This could also be the reason for delayed sexual maturity as reported in case of Kerela poisoning.

Histopathological changes such as atrophy and decreased number of germ cells may be a reason for decreased testicular weights as observed in the present and earlier reports (29, 35,36) or it can be due to increased protein breakdown due to endosulfan (37).

Such pathological changes can be attributed to oxidative stress induced by endosulfan as reported in earlier studies (6,13, 38, 39). Moreover, overproduction of reactive oxygen species damages vital components of cell, like nucleic acids and proteins which further lead to oligozoospermia and abnormal spermatozoa (29).

Vitamin E supplementation resulted in improvement of the structural alterations in the testis due to endosulfan. This amelioration could be attributed to the capacity of vitamin E to scavenge ROS and reduce the oxidative stress as reported in earlier studies (17, 40)

Thus, on the basis of above findings it can be concluded that pretreatment with vitamin E ameliorates the severe pathological alterations in the testis due to endosulfan exposure. Moreover, it can be said that if occupationally exposed workers supplement their diet with vitamin E the endosulfan induced toxicity could be minimized.

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